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**PCT** WORLD ORGANIZATION FOR INTELLECTUAL PROPERTY
International Office
INTERNATIONAL APPLICATION PUBLISHED IN ACCORDANCE WITH
THE INTERNATIONAL PATENT COOPERATION TREATY (PCT)

(51) **International patent classification[6]:**
**A61K 9/14, C07K 14/785**

(11) **International publication number: WO 97/26863**

(43) **International publication date:** July 31, 1997

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(21) **International application number:** PCT/EP97/00230

(22) **International application date:** January 18, 1997

(30) **Priority data:**

196 02 332.7 January 24, 1996 DE

96103442.8 March 6, 1996 EP

*(34) countries for which the regional or international application has been filed:* DE, etc.

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(81) **Designated countries:** AL, AU, BA, BG, BR, CA, CN, CZ, EE, GE, HU, IL, JP, KR, LT, LV, MK, MX, NO, NZ, PL, RO, SG, SI, SK, TR, UA, US, Eurasian Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE)

**Published**

*With an international search report.*

*Prior to the expiration of the deadline that is permitted for changes in the claims.*

*Publication will be repeated in the event that changes arise.*

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(54) **Title:** PROCESS FOR THE PRODUCTION OF POWDER-TYPE PULMONARY SURFACTANT PREPARATIONS

(57) **Summary**

A process is described for the production of a powder-type pulmonary surfactant preparation containing hydrophobic pulmonary surfactant proteins that is characterized by the feature that an organic solution or suspension, which contains hydrophobic pulmonary surfactant proteins and, if desired, additional components, is subjected to spray drying. Powder preparations are obtained that exhibit very good storage stability and that are easy to reconstitute and that are also suitable for administration by inhalation.

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# Process for the production of powder-type pulmonary surfactant preparations

## Technical area

The invention pertains to a process for the production of powder-type pulmonary surfactant preparations.

## Prior art

The lungs of all vertebrates contain a mixture of substances that is termed a "pulmonary surfactant". It exhibits surface active properties and decreases the surface tension in the alveolar region of the lungs sufficiently far that collapse of the final respiratory tract regions is avoided when exhaling. This mixture of substances regulates the surface tension dynamically so that the collapse of the small alveoli in favor of the larger ones, which is to be expected in accordance with Laplace's law, is avoided by appropriate adaptation of the surface tension. As a result, a well balanced histologically and physiologically stable structure of the lungs is produced in this way.

The pulmonary surfactant is secreted by the type II alveolar pneumocytes in the form of lamellar bodies. These are compact units comprising phospholipid double layers (bi-layers) with a high proportion of dipalmitoylphosphatidylcholine (DPPC) and phosphatidylglycerine (PG). Proteins are contained in the pulmonary surfactant as additional essential components and these are designated SP-A, SP-B and SP-C. SP-A is a high molecular glycoprotein that plays a decisive role in secretion regulation.

The hydrophobic proteins SP-C and, to a lesser extent, SP-B take on the role of "thermodynamic catalysts" for the formation of the monomolecular surface film (the surfactant in the narrower sense). The spreading kinetics are enormously accelerated as a result of the presence of these proteins. It is only as a result of this that the delay-free adaptation of the surfactant composition to the surface tension requirements in question becomes possible. These properties are reflected

in the extremely hydrophobic character of the proteins, especially those of SP-C.

In the case of premature babies, their lungs are not yet capable, or are not yet capable to an adequate extent, of producing pulmonary surfactant and this leads to a life-threatening shortage of oxygen (Infant Respiratory Distress Syndrome, IRDS). IRDS represents the main cause of death among premature babies.

For many years, it has proven expedient to treat IRDS by introducing pulmonary surfactant preparations into the lungs of the affected children. It is known from pilot studies that pulmonary surfactant preparations are also clinically effective in cases of ARDS (Adult Respiratory Distress Syndrome) including ALI (Acute Lung Injury).

Pulmonary surfactant preparations can be recovered from the lungs of animals in an expensive extraction and centrifugation process (pulmonary lavage) or they can be assembled from the individual components.

WO 92/06703 describes the production of synthetic pulmonary surfactant preparations by evaporating a solution, in chloroform, that contains phospholipids, such as dipalmitoylphosphatidylcholine (DPPC) and dioleylphosphatidylethanolamine (DOPE), and cholesterol to give a thin film with use being made of a rotary evaporator, whereby this film is re-suspended in a buffer together, optionally, with suitable proteins.

It is known from WO 91/00871 that an organic solution of a pulmonary surfactant preparation, which contains a pulmonary surfactant protein that has been produced by means of genetic engineering, can be concentrated by evaporation and rehydrated with a buffer and then lyophilized. The lyophilisate that is obtained has the disadvantage that it has to be rehydrated for 15 minutes at 37°C prior to administration and this is very tiresome and error-prone for the user.

A process for the production of a pulmonary surfactant preparation is indicated in EP 0119056 in which all the components are dissolved in an organic solvent and the solution that is obtained is evaporated to dryness under reduced pressure and the residue that is obtained is re-suspended in

an aqueous medium over an extended period of time at an elevated temperature and the suspension that is obtained is subjected to freeze drying. This process is also very expensive industrially.

DE 3229179 discloses a process for the production of a protein-free pulmonary surfactant preparation in which the components are dissolved in glacial acetic acid and the solution that is obtained is freeze dried. A disadvantageous aspect of this process is the use of glacial acetic acid because it necessitates extensive safety procedures.

The proposal is made in EP 0655237 that medicinal drug preparations, which are required to be administered via inhalation in the form of a suspension aerosol, be produced by spray drying from ethanol/water mixtures. This process is described as being suitable, inter alia, for compositions that contain hydrophilic proteins, such as e.g. ecatibant acetate, human insulin and buserelin acetate.

Specification of the invention

The problem, which forms the basis of the present invention, is seen to comprise the indication of a process for the production of protein-containing, powder-type, pulmonary surfactant preparations, which contain hydrophobic pulmonary surfactant proteins, whereby this process is as inexpensive as possible industrially and leads to a product that is stable on storage and that can be applied with advantage.

Surprisingly, it has now been found that this problem can be solved by subjecting an organic solution or suspension, which contains hydrophobic pulmonary surfactant proteins and, optionally, additional components, to spray drying.

In accordance with this process, a product is obtained that is stable during storage over a long period of time and that can be re-suspended, prior to application, without particular expense. Its small particle size (1 to 5 μm) is to be emphasized as a special advantage of the powder that is obtained, whereby this permits administration via inhalation. This aspect is of special

significance in the case of using pulmonary surfactant preparations as a carrier for medicinal drugs that are capable of being applied via the lungs.

The way in which the components of pulmonary surfactants, some of which are highly temperature-sensitive, withstand the conditions of the spray drying process is quite astounding and has been inexplicable thus far. Thus, for example, it is known that the pulmonary surfactant protein SP-C aggregates very rapidly above -20°C, and thereby becomes inactivated. By contrast, this protein withstands the spray drying process in accordance with the invention with no noteworthy decomposition, and it is then present in the form of a loose powder that is stable during storage at room temperature.

Thus the subject of the invention is a process that is characterized by the feature that an organic solution or suspension, which contains hydrophobic pulmonary surfactant proteins and, optionally, additional components, is subjected to spray drying.

Additional subjects will be seen from the patent claims.

As far as the hydrophobic pulmonary surfactant proteins are concerned, consideration can be given both to proteins of natural origin as well as to those that have been produced synthetically, including those that have been produced by genetic engineering, especially SP-B and SP-C, along with their mixtures. The term synthetic proteins is also to be understood to mean those proteins whose amino acid sequence deviates, more or less markedly, from the amino acid sequence of naturally occurring pulmonary surfactant proteins, including those synthetic proteins that have an amino acid sequence that has been designed completely independently of their pulmonary surfactant properties in the way in which these have been described in EP 0593094 and EP 92/22315, for example. These proteins can be isolated, synthesized and purified by known processes.

The following are suitable as solvents for the production of an organic solution or suspension in accordance with the invention: alcohols, such as methanol, ethanol, 1-propanol, 2-propanol and the butanols; chlorohydrocarbons, such as dichloromethane, chloroform, etc.; acetone, ether,

hydrocarbons [e.g.] benzene and toluene, and their mixtures, whereby water can also be contained therein provided that miscibility with water is ensured. The maximum water content amounts to 25% by weight. A water content of 5 to 15% by weight is preferred. On the basis of his technical knowledge in the area of spray drying and, if necessary, by means of conventional experiments, it is an easy matter for the technical expert to select the solvents or, as the case may be, solvent mixtures that are best suited to the surfactant mixtures that are to be dried.

The pulmonary surfactant preparations contain conventional substances such as, in particular, phospholipids, carboxylic acids and buffer substances as further components.

The solution can be filtered through a sterile filter prior to the start of spray drying. The spray drying process takes place in a way that is known as such. A comprehensive presentation of this technique is found in the publications by K. Masters: Spray Drying Handbook, 5th Ed. 1991 and by J. Broadhead, S.K. Edmond Ronan and C.T. Rhodes: The Spray Drying of Pharmaceuticals, Drug. Dev. Ind. Pharm. 18, 1169 (1992). The principle of spray drying comprises subdividing a solution or suspension of the product, which is to be dried, into fine droplets and drying these with a stream of hot gas. The proportion of solids, which remains behind after evaporating the solvent, is separated from the stream of gas by means of a cyclone-type dust separator and/or through a filter unit and collected.

In accordance with the invention, it has proven expedient to use alcohols and chlorohydrocarbons as solvents, especially methanol, ethanol, 2-propanol and chloroform and mixtures thereof, optionally with a small addition of water (up to maximally 25% by weight). Air and nitrogen can be considered, in particular, as the gases that are used for drying.

Temperatures of 60 to 200°C or, preferably, 90 to 150°C are expedient for the gas inlet temperature. The gas outlet temperature is maintained at 40 to 80°C or, preferably, 50 to 70°C by means of appropriate control of the spraying intensity and/or the quantity of gas.

Preparation examples

**Example 1**

7.0 g of 1,2-dipalmitoyl-3-sn-phosphatidylcholine, 2.5 g of sodium 1-palmitoyl-2-oleoyl-3-sn-phosphatidylglycerol, 205 mg of calcium chloride dihydrate and 250 mg of palmitic acid are dissolved in 300 ml of ethanol/water (85:15) with heating to 60°C and then they are cooled to room temperature and mixed with 350 ml of a solution of SP-C in 9:1 chloroform/methanol (c = 429 mg/ml). The resulting solution is spray dried in a Büchi B 191 laboratory spray drier. The spraying conditions are as follows. Drying gas: air; inlet temperature: 90°C; outlet temperature: 52-54°C. A loose powder is obtained.

**Example 2**

A solution, in chloroform/methanol, of surfactant that had been recovered from the lungs of cattle (obtained by extraction and the purification steps that are described in e.g. EP 406732) is spray dried under the following conditions: Büchi B 191 laboratory spray drier; drying gas: nitrogen; inlet temperature: 80°C; outlet temperature: 50-52°C. A fine yellowish powder is obtained.

**Example 3**

10.95 g of 1,2-dipalmitoyl-3-sn-phosphatidylcholine, 4.6 g of ammonium 1-palmitoyl-2-oleoyl-3-sn-phosphatidylglycerol, 418 mg of calcium chloride dihydrate and 750 mg of palmitic acid are dissolved in 330 ml of 2-propanol/water (85:15) at 50°C and then, after cooling to 30°C, they are mixed with 620 ml of a solution of SP-C in isopropanol/water (95:5; c = 484 mg/ml). The resulting solution is spray dried in a Büchi B 191 laboratory spray drier. The spraying conditions are as follows. Drying gas: nitrogen; inlet temperature: 100°C; outlet temperature: 58-60°C. A colorless powder is obtained.

**Example 4**

3.74 g (5.1 mmol) of 1,2-dipalmitoyl-3-sn-phosphatidylcholine, 2.81 g (3.7 mmol) of 1-palmitoyl-2-oleoyl-3-sn-phosphatidylcholine, 2.90 g (3.9 mmol) of sodium 1,2-dipalmitoylphosphatidyl-3-sn-phosphatidylglycerol, 234 mg of palmitic acid and 279 mg (1.9 mmol) of calcium chloride dihydrate are dissolved in 160 ml of 2-propanol/water (85:15) at 50°C and then, after cooling to 30°C, they are mixed with 566 ml of a solution of SP-C in isopropanol/water (92:8; c = 330 mg/ml) at 30°C. The resulting solution is spray dried in a Büchi B 191 laboratory spray drier. The spraying conditions are as follows. Drying gas: nitrogen; inlet temperature: 90°C; outlet temperature: 58-60°C. A colorless powder is obtained.

**Example 5**

0.5 g of RLLLLRLLLLRLLLLRLLLLR (R = Arg, L = Leu), 7.125 g of 1,2-dipalmitoyl-3-sn-phosphatidylcholine and 2.43 g of ammonium 1-palmitoyl-2-oleoyl-3-sn-phosphatidylglycerol are dissolved in 500 ml of 1:1 chloroform/methanol with heating at 45°C and then they are spray dried in a Büchi B 191 laboratory spray drier. The spraying conditions are as follows. Drying gas: nitrogen; inlet temperature: 85°C; outlet temperature: 55°C. A colorless powder is obtained.

## Patent claims

1. Process for the production of a powder-type pulmonary surfactant preparation containing hydrophobic pulmonary surfactant proteins, characterized by the feature that an organic solution or suspension, which contains hydrophobic pulmonary surfactant proteins and, if desired, additional components, is subjected to spray drying.

2. Process in accordance with Patent Claim 1, characterized by the feature that, as the hydrophobic pulmonary surfactant proteins, SP-C and/or SP-B are present in the organic solution or suspension.

3. Process in accordance with Patent Claim 2, characterized by the feature that SP-C is present in the organic solution or suspension.

4. Process in accordance with Patent Claim 1, characterized by the feature that the organic solution or suspension contains 5 to 15% by weight of water.

5. Process in accordance with Patent Claim 1, characterized by the feature that phospholipids are contained therein as additional components.

6. Process in accordance with Patent Claim 1, characterized by the feature that spray drying is carried out in a heated gas.

7. Process in accordance with Patent Claim 6, characterized by the feature that air or nitrogen is used as the gas.

8. Process in accordance with Patent Claim 6, characterized by the feature that the gas has an inlet temperature of 60 to 200°C and an outlet temperature of 40 to 80°C.

9. Process in accordance with Patent Claim 7, characterized by the feature that the gas has an inlet temperature of 90 to 150°C and an outlet temperature of 50 to 70°C.

10. Powder-type pulmonary surfactant preparations that are obtained using the process in accordance with Patent Claims 1 through 9.

# INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/EP 97/00230

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/14 C07K14/785

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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